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Biochemistry and Biophysics of the Developing Hen's Egg

I. Influence of Humidity

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BY

ALEXIS L. ROMANOFF



ITHACA, N. Y.

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OF THE DEVELOPING HEN'S EGG

I. INFLUENCE OF HUMIDITY

ALEXIS L. ROMANOFF

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BIOCHEMISTRY AND BIOPHYSICS OF THE DEVELOPING HEN'S EGG¹

I. INFLUENCE OF HUMIDITY

ALEXIS L. ROMANOFF

The effect of humidity on the chick embryo was perhaps first studied scientifically by Réaumur as early as 1749. His experimental observations show that excessive humidity frequently gives rise to pathological forms, although some embryos complete their development and reach the state of hatching without manifesting any physical defects. Réaumur observed also that the egg contents produced a curious phenomenon: the albumen absorbed moisture from the air, became watery, and, instead of drying as when normal moisture prevails, oozed through the shell. This "liquefaction of albumen," as Réaumur termed it, made an obstruction to hatching. Some chicks, he found, had commenced to pip through the shell, but their beaks, being only partially outside, had collected enough of the exuding "albumen" to produce death through hindrance of respiration. Other chicks had become so swathed in linings or membranes as to prevent their freeing themselves and breaking the shell.

More than a century later, Dareste (1882) observed that excessive humidity facilitates the growth of molds (*Aspergillus*) in the albumen; and when abundant, these molds are sufficient to hinder the development of the embryo.

These early studies of high humidity in incubation were limited to incidental researches on the production of monsters. The effect of low as well as of high humidity on growth, metabolism, and mortality of the embryo at successive stages of incubation was not studied, either physiologically or chemically.

In modern practice of artificial incubation, however, attempts have been made to establish the optimum humidity condition by mere observation of the number of chicks hatched, and it has been noticed that the best hatches are obtained at about 55 to 65 per cent relative humidity. Whether or not this humidity should be uniform throughout the incubation period, has not been adequately determined.

In the present investigation the problem was approached by the study of comparative growth, metabolism, and mortality of the embryo, and

¹Contribution from the Laboratory of Experimental Embryology of the Department of Poultry Husbandry, Cornell University Agricultural Experiment Station, Ithaca, New York.

of physicochemical changes in eggs and egg contents throughout the incubation period, under optimum and extreme humidity conditions in the incubator. The optimum humidity was chosen as 60 per cent, and the two extreme humidities as 40 and 80 per cent. As the writer has observed in a number of cases, such extreme conditions still exist in our commercial incubators. It is therefore of practical value to determine what is the effect of such fluctuations of humidity on the life process of the developing embryo.

MATERIAL USED

The eggs used were all from the same commercial flock of White Leghorn hens. They were collected within three or four days, and were kept at 10° C. prior to the incubation.

In order to avoid seasonal variation in the quality of the eggs, all experiments were conducted during winter months (December, 1927, to March, 1928), when hens are confined in houses and are kept under more or less uniform feeding and management.

In selecting the eggs, the primary consideration was uniformity of size, shape, and shell texture, in order to minimize possible variation in the hatching quality of the eggs due to their physical properties (Romanoff, 1929 b). The average original weight of the eggs was 60.9 ± 2.9 grams, and the average shape — that is, the ratio of length to width — was 1.41 ± 0.09 . The shell texture was determined by candling and by observing the external condition of the egg.

EQUIPMENT

The incubation was carried on in a special electric laboratory incubator (Romanoff, 1929 a) having a capacity of 160 eggs. This incubator, shown diagrammatically in figure 1, has been so adapted that each of the four important factors of incubation — temperature, humidity, quality of air, and movement of eggs — can be independently and accurately controlled.

The paths of possible air flow through the incubator, under various methods of control of the environmental conditions, are shown in figure 1 by arrows. In general, outdoor fresh air, regardless of weather condition, is drawn by the blower (3) through the room-heating coil (1), the humidifier (9), and the incubator-heating coil (10), and then, fully conditioned in respect to temperature and humidity, through the egg chamber.

The heat is supplied by the electric heater (11), regulated by the thermostat (12), and recorded by the thermograph (15) and the thermometers (a_1 , b_1 , c_1 , d_1 , e_1 , and f_1). The moisture is supplied and regulated by the humidifier (9), and recorded by the hygrometer (14).

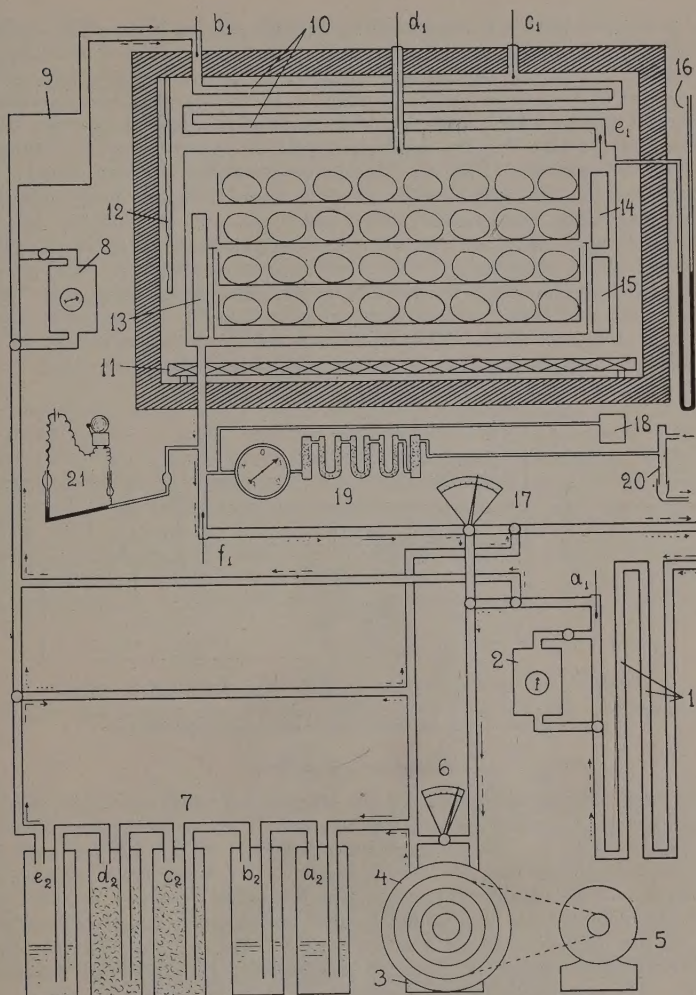


FIGURE 1. DIAGRAM OF THE LABORATORY ELECTRIC INCUBATOR USED IN THE STUDY OF COMPARATIVE GROWTH, METABOLISM, AND MORTALITY OF THE CHICK EMBRYO UNDER VARIOUS ENVIRONMENTAL CONDITIONS OF INCUBATION

The different types of arrows show the paths of air flow through the incubator for each of the three ventilation systems: the open system, $\cdots \rightarrow$; the semi-closed system, $--- \rightarrow$; and the closed system, $\longrightarrow$

The quality of the air is regulated by changing from internal to external or reverse ventilation at the valve (17), with the aid of the absorption system (7: a_2 , b_2 , c_2 , d_2 and e_2) adapted to the use of in-going or out-going air; and is determined in respect to the carbon-dioxide content either by the residual system (19), gravimetrically, using the water pump (20), or by the Haldane gas-analysis apparatus (18), volumetrically, or by both. The ventilation is provided by the blower (3), driven by the electric motor (5); is regulated by both the cone pulley (4) and the by-pass valve (6); and is recorded by the gas meters (2) and (8), for external and internal ventilation, respectively. Warning of a failure of the ventilation is given by an electric alarm (21) connected with the living quarters of the operator. Accidental variation in air pressure in the egg chamber is indicated by the water monometer (16). The movement of eggs is controlled by the electric turning device (13), shown diagrammatically in figure 2.

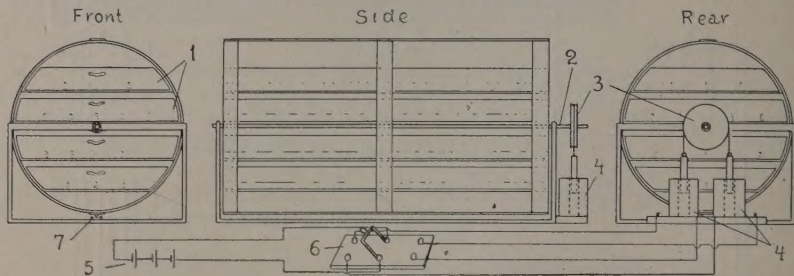


FIGURE 2. DIAGRAM OF EGG TRAYS WITH ELECTRIC TURNING DEVICE

1, Egg trays; 2, main shaft; 3, pulley; 4, electromagnets; 5, wet B batteries; 6, double-pole, double-throw, knife switch; 7, lock for keeping egg trays in horizontal position

METHODS OF STUDY

The eggs were incubated under the standard conditions of all physical factors except humidity, which was variable. The average temperature was $38.0^{\circ} \pm 0.2^{\circ}$ C.; the average humidity was 60.0 ± 1.0 per cent for optimum condition, and 80.2 ± 0.5 and 40.8 ± 1.0 per cent for extreme conditions. The inside ventilation was 0.5 cubic foot per minute, and fresh air from outside was added to give an adequate supply of oxygen. The carbon-dioxide content was increased from 0.15 to 0.65 per cent, similar to that found in natural incubation (Lamson and Edmond, 1914). The eggs were turned regularly three times a day.

At the beginning of incubation an allowance of two hours was made to heat the eggs up to the physiological zero, about 21° C. (Edwards,

1902). Then, at intervals of twenty-four hours, all the incubated eggs were candled for dead germs and were weighed collectively on a Sartorius balance to 0.01 gram, and four eggs with normally developed embryos were removed for study.

The eggs having an embryo sufficiently large for analysis (at least three days of age) were opened at the blunt end. Pieces of the various parts were removed: first, the shell with the shell membranes, then the vitelline membrane, and, when present, the allantoic and amnionic sacs. With forceps and a pair of scissors, the embryo was gently separated from the yolk sac at the proximal end of the umbilical cord, then dropped into a vessel containing distilled water, cleaned from adhering membranes, and washed once more in another vessel of distilled water. Finally, the embryo was placed in a porcelain crucible, drained with a pipette, and weighed on the analytical balance.

A portion of albumen and yolk, and a whole eggshell with shell membranes, were separately collected for analysis. In collecting fresh albumen, precautions were taken to have a uniform sample of each of the three layers—outer, middle, and inner—which contain, respectively, 11.59, 12.45, and 14.55 per cent of dry matter (Romanoff, 1929 c.)

Dry matter of the embryo, yolk, albumen, and eggshell with shell membranes, was determined by drying to constant weight in a Freas electric vacuum oven at a temperature of 80° C. and a vacuum of at least 63.5 centimeters (25 inches). The oven used has a special arrangement, shown diagrammatically in figure 3.

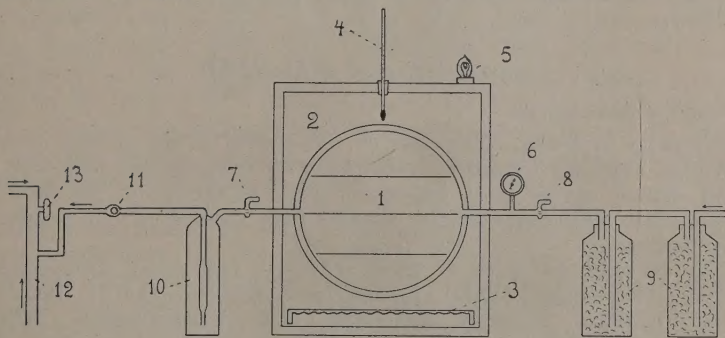


FIGURE 3. DIAGRAM SHOWING ARRANGEMENT OF APPARATUS USED FOR DETERMINING THE DRY MATTER IN THE EMBRYO AND THE EGG CONTENTS

- 1, Vacuum drier chamber; 2, heating chamber; 3, heating element controlled by thermostat; 4, thermometer; 5, pilot bulb; 6, vacuum gage; 7 and 8, air valves; 9, calcium-chloride drying tubes; 10, washing bottle, used to prevent back flow of water; 11, one-way ball valve; 12, water pump; 13, water valve

Total ash of the embryo and the egg contents was estimated by heating the dry matter to a white ash in a Muffle electric furnace at a dull red heat, indicating approximately 720° to 750° C.

Calcium, as calcium oxide (CaO), in embryo and egg contents, was estimated gravimetrically from the total ash by the modified McCrudden method (Romanoff, 1928). In this analysis, shaking was performed with the aid of a special "swinging type" of shaking machine designed in this laboratory (Romanoff, 1930 a).

EXPERIMENTAL RESULTS

The experimental data of this study are here grouped according to the influence of humidity on the embryo, direct and indirect. Under direct influence the following factors are considered: growth, growth cycles, metabolism (of calcium), and mortality of the embryo. All factors under indirect influence of humidity are closely associated with the physicochemical condition of the egg contents surrounding the embryo.

The optimum humidity condition, 60 per cent, is taken at present as a standard for the artificial incubation (Romanoff, 1930 b). Therefore the results obtained under this condition are presented here and are used henceforth for comparison with the results obtained under the extreme conditions, 80 and 40 per cent relative humidity (Romanoff, 1929 d).

Direct influence of humidity

Growth of the embryo

Optimum humidity. The data on the growth of the embryo under optimum humidity condition (table 1), with selected eggs and with a good control of the most important physical factors of incubation, demonstrate much more regular changes than those shown by previous investigators.

For example, the data of Falck (Preyer, 1885:510), Liebermann (1888), Hasselbalch (1900), Lamson and Edmond (1914), Murray (1925), Mitchell, Hamilton, and Card (1926), Schmalhausen (1926), Henderson and Brody (1927), and Needham (1927), representing the normal embryonic growth under the standard conditions of incubation, are difficult to compare. Their inconsistency is due to differences in: (1) the breed of fowls, because the growth curve of the embryo is specific to its species; (2) the size of eggs, as the size of an egg corre-

TABLE 1. GROWTH OF THE CHICK EMBRYO UNDER OPTIMUM HUMIDITY

Stage of incubation	Body wet weight	Gain in wet weight (W ₂ -W ₁)	Percentage gain in wet weight $\frac{(W_2-W_1)}{W_1} 100$	Dry-matter content	Total ash content	Calcium (CaO) content
(Days)	(Grams)	(Grams)	(Per cent)	(Grams)	(Grams)	(Grams)
3.....	0.0185 ± 0.0012*	0.0293	158.38	0.0016 ± 0.0007		
4.....	0.0478 ± 0.0081	0.0808	169.04	0.0032 ± 0.0010		
5.....	0.1286 ± 0.0350	0.1664	129.39	0.0099 ± 0.0017		
6.....	0.295 ± 0.047	0.279	94.58	0.0178 ± 0.0029		
7.....	0.574 ± 0.022	0.578	100.70	0.0358 ± 0.0018	0.0061 ± 0.0004	
8.....	1.152 ± 0.095	0.375	32.55	0.0743 ± 0.0062	0.0106 ± 0.0009	
9.....	1.527 ± 0.097	0.736	48.20	0.1011 ± 0.0065	0.0131 ± 0.0010	
10.....	2.263 ± 0.181	1.421	62.79	0.2044 ± 0.0092	0.0204 ± 0.0011	
11.....	3.684 ± 0.356	1.384	37.57	0.284 ± 0.033	0.0380 ± 0.0042	
12.....	5.068 ± 0.396	2.306	45.50	0.423 ± 0.041	0.0507 ± 0.0060	
13.....	7.374 ± 0.987	2.368	32.11	0.694 ± 0.114	0.0741 ± 0.0155	
14.....	9.742 ± 0.569	2.302	23.63	1.072 ± 0.063	0.1026 ± 0.0046	0.0217 ± 0.0009
15.....	12.044 ± 0.683	3.936	32.68	1.568 ± 0.149	0.1463 ± 0.0148	0.0311 ± 0.0034
16.....	15.980 ± 0.759	2.610	16.33	2.497 ± 0.068	0.2143 ± 0.0082	0.0513 ± 0.0054
17.....	18.590 ± 1.310	3.241	17.43	3.246 ± 0.176	0.2662 ± 0.0182	0.0683 ± 0.0066
18.....	21.831 ± 2.297	3.785	17.34	3.884 ± 0.330	0.3280 ± 0.0337	0.0988 ± 0.0276
19.....	25.616 ± 1.882	4.589	17.91	4.522 ± 0.207	0.4096 ± 0.0185	0.1116 ± 0.0018
20.....	30.205 ± 0.631			5.384 ± 0.078	0.4975 ± 0.0147	0.1331 ± 0.0066

* The average variation is calculated from the formula $\frac{(a-x) + (b-x)}{4}$, in which *a* is the heaviest embryo, *b* is the lightest, and *x* is the average of four embryos.

sponds to the size of the embryo presumably in all stages of the embryonic development; (3) the individual quality of eggs, which may influence the growth of the embryo by some factors inherited from one hen or a flock of hens; (4) the climatic conditions; (5) the season of the year; (6) the make-up of the incubator in respect to the control of physical factors of incubation; and (7) the local practice and custom of hatching in a given section of the country or in a given part of the world.

Similar inconsistency is found with the data of Murray (1926), Mitchell, Hamilton, and Card (1926), Schmalhausen (1926), and Need-

ham (1927) on dry matter, and with those of Liebermann (1888) and Murray (1926) on total ash content, of the embryo.

The calcium content of the embryo has been given by Plimmer and Lowndes (1924). The value given by them is very low, only 0.1262 gram on the twenty-first day of incubation, while ours under optimum humidity condition is 0.1559, excluding the yolk sac.

Extreme humidity. The growth of the embryo under high and under low humidity, as shown by the body wet weight and by the contents of dry matter, total ash, and calcium (table 2), is quite distinct from

TABLE 2. GROWTH OF THE CHICK EMBRYO UNDER EXTREME HUMIDITY

Stage of incubation (Days)	High humidity				Low humidity			
	Body wet weight	Dry matter in wet weight	Total ash in dry matter	Calcium (CaO) content	Body wet weight	Dry matter in wet weight	Total ash in dry matter	Calcium (CaO) content
	(Grams)	(Per cent)	(Per cent)	(Grams)	(Grams)	(Per cent)	(Per cent)	(Grams)
3.....	0.012 ± 0.003	9.48			0.013 ± 0.006	12.87		
4.....	0.089 ± 0.030	4.94			0.043 ± 0.002	6.24		
5.....	0.225 ± 0.027	5.66			0.138 ± 0.029	7.02		
6.....	0.429 ± 0.046	5.97			0.295 ± 0.076	6.60		
7.....	0.672 ± 0.151	6.23	19.21		0.611 ± 0.106	6.49	14.69	
8.....	1.258 ± 0.043	6.11	16.44		0.977 ± 0.075	6.51	13.00	
9.....	1.921 ± 0.123	6.28	14.55		1.451 ± 0.091	6.74	10.21	
10.....	2.752 ± 0.068	7.26	15.20		2.147 ± 0.126	7.14	11.26	
11.....	3.968 ± 0.341	7.70	15.70		4.232 ± 0.366	8.43	11.01	
12.....	5.315 ± 0.362	8.18	13.94		5.455 ± 0.392	8.72	11.92	
13.....	8.072 ± 0.551	9.82	12.63		7.385 ± 0.311	12.29	9.09	
14.....	10.741 ± 0.871	11.00	11.15	0.0300	9.450 ± 0.551	13.23	8.97	0.0293
15.....	14.329 ± 0.727	13.26	10.17	0.0466	12.873 ± 0.713	14.14	8.79	0.0398
16.....	15.505 ± 0.558	15.04	9.17	0.0833	14.124 ± 0.772	14.88	9.08	0.0513
17.....	20.551 ± 2.038	16.74	8.69	0.1235	16.790 ± 0.935	17.84	8.11	0.0688
18.....	21.945 ± 2.081	17.31	8.90	0.1368	19.310 ± 1.969	18.02	8.52	0.0762
19.....	26.253 ± 3.191	17.94	8.92	0.1379	20.462 ± 2.471	18.29	8.67	0.0897
20.....	26.235 ± 1.827	17.66	9.07	0.1410	26.287 ± 1.898	18.05	8.83	0.1187
21.....	31.140 ± 0.969	20.30	8.73	0.1623	29.572 ± 3.631	22.54	7.72	0.1363

that under optimum humidity. It is seen in figure 4 that the growth under either extreme condition is irregular and that it is noticeably retarded under low humidity. The hatching time came one day earlier under optimum than under extreme conditions.

The dry matter and the total ash content corresponded closely to the weight of the embryo in all cases. As shown in table 2, there is less dry matter in wet weight under high than under low humidity, but more total ash in dry matter. These conditions indicate that perhaps under high humidity the embryo has somewhat greater moisture content, yet more of total ash per unit of dry matter and possibly in the whole organism, than under low humidity.

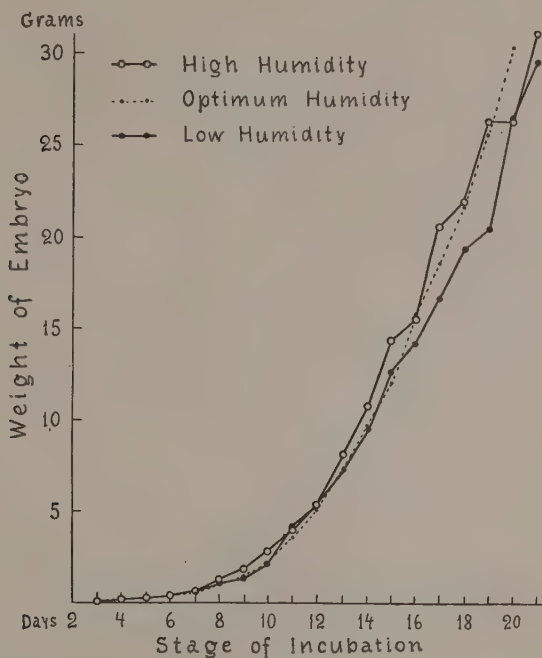


FIGURE 4. CUMULATIVE GROWTH CURVES OF THE EMBRYO UNDER OPTIMUM AND UNDER EXTREME HUMIDITY

Calcium (CaO) content of the embryo, which is a part of the total ash, is found to be greater under high than under low humidity. This may be attributed mainly to rapid assimilation of calcium by the embryo at high humidity in the incubator. The curves for both extreme conditions are more irregular than that for the optimum condition (figure 5). The curve under high humidity declines after eighteen days, which means simply that high humidity might be favorable, but only during a limited period of the embryonic development, and this period has yet to be determined experimentally.

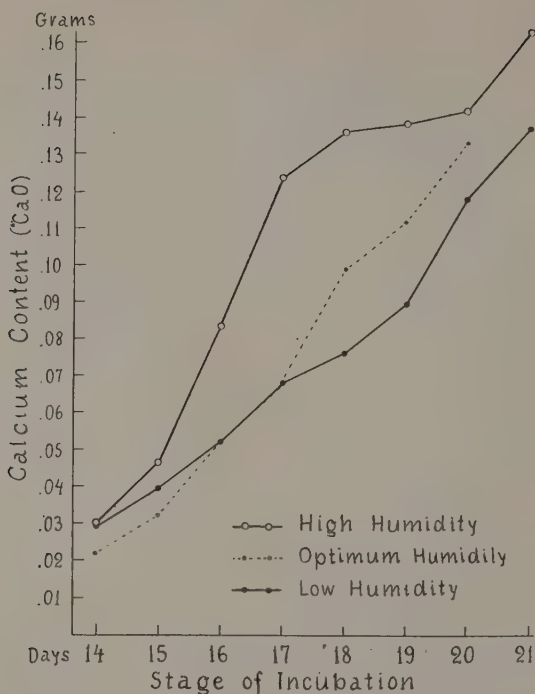


FIGURE 5. CALCIUM CONTENT OF THE EMBRYO UNDER OPTIMUM AND UNDER EXTREME HUMIDITY

Cycles in the growth of the embryo

Optimum humidity. In spite of the extremely variable data on the growth of the embryo found by the above-mentioned investigators, the normal growth cycles, which have been observed in the life span of the embryo (Romanoff, 1929 e), are well demonstrated in all cases. Our data on the daily gain in weight of the embryo closely approximates the average curve plotted from the data of previous investigators (figure 6). These growth cycles are three in number, and are indicated by retardation periods in the growth at about nine, sixteen, and twenty days of incubation.

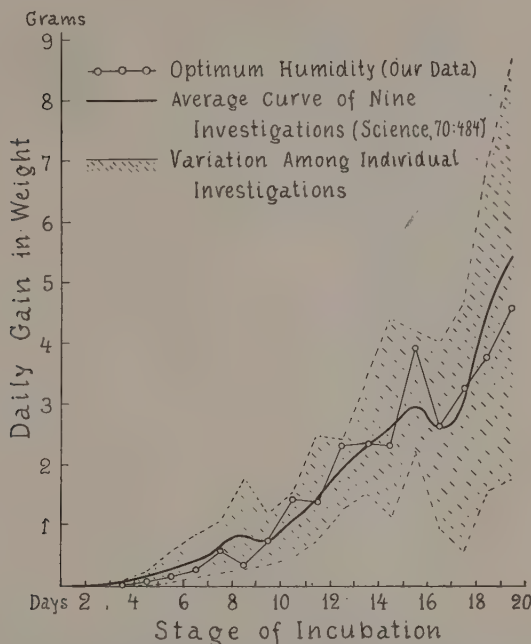


FIGURE 6. GROWTH CYCLES UNDER OPTIMUM HUMIDITY, SHOWN BY THE DAILY GAIN IN WEIGHT OF THE EMBRYO

Extreme humidity. The growth cycles of the embryo which were found under the optimum condition are almost absent under extreme-humidity conditions. The irregularity in the growth, as shown by the daily gain of body weight and by the contents of dry matter and total ash (figure 7), is pronounced under either extreme from about fourteen days of incubation, and is much greater under high than under low humidity.

Calcium metabolism

Optimum humidity. In the past the most striking disagreement has been found regarding calcium metabolism of the embryo. Some investigators, among them Voit (1877) and Pott and Preyer (1882), even

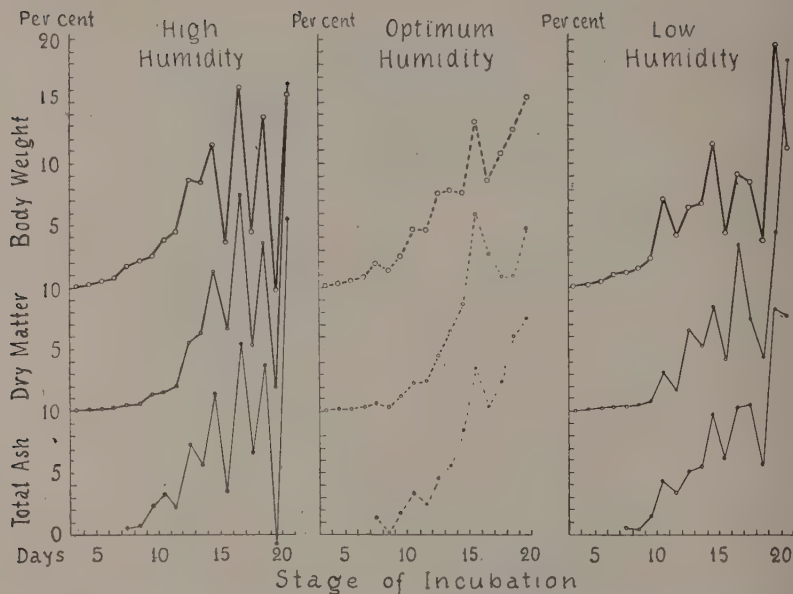


FIGURE 7. GROWTH CYCLES OF THE EMBRYO UNDER OPTIMUM AND UNDER EXTREME HUMIDITY, SHOWN BY THE DAILY GAIN IN WEIGHT, AND BY THE CONTENTS OF DRY MATTER AND TOTAL ASH, EXPRESSED IN PERCENTAGES AFTER THE FORMULA $\frac{W_2 - W_1}{W_n} \cdot 100$

(W_1 =gain in weight for preceding day; W_2 =gain in weight for following day; W_n =sum of daily gains)

failed to prove that the chick embryo uses the calcium from the shell; while others—Prout (1822), Vaughan and Bills (1878–79), Carpioux (1908), and Plimmer and Lowndes (1924)—furnished some evidence to demonstrate it. Our data, based on the average of four daily observations, give a smooth curve for the increase of calcium in the embryo (figure 5). At the time of hatching, the embryo alone, without the yolk sac, has about 0.16 gram, while in the contents of a fresh egg there was found only 0.025 gram. The additional calcium in the embryo, the yolk sac, and possibly other parts of the egg contents, such as the shell membrane (Plimmer and Lowndes, 1924), came by the process of metabolism during the incubation period from but one source, the calcareous eggshell.

Extreme humidity. It has been shown (table 2 and figure 5) that the daily increase of calcium in the embryo is greater under high than under low humidity. In spite of a decline in the curve of calcium content during the second critical period, the difference in favor of high humidity is still noticed at the time of hatching (table 3). The ques-

TABLE 3. CALCIUM METABOLISM OF THE CHICK EMBRYO UNDER EXTREME HUMIDITY

	Calcium (CaO) content	
	High humidity	Low humidity
	(Grams)	(Grams)
Incubated eggs (21 days):	(4)	(4)
Number of analyses.....		
Embryo.....	0.1623 ± 0.0135	0.1362 ± 0.0088
Yolk sac.....	0.0555 ± 0.0105	0.0582 ± 0.0089
Total.....	0.2178	0.1944
Unincubated eggs:	(8)	
Number of analyses.....		
Yolk.....	$0.0189 \pm 0.0073^*$	
Albumen.....	$0.0065 \pm 0.0026^*$	
Total.....	0.0254	
Total increase during incubation.....	0.1924	0.1690

* These values are variable with such factors as season, individual flock, or individual hen, and possibly some others.

tion has not been studied, but it is quite possible that a chick hatched from this group would have larger skeletal structure or a more advanced stage of skeletal ossification than one from the group under low-humidity condition.

Mortality

Optimum humidity. Under both natural and artificial methods of incubation, it has been observed that the chick embryo during its development normally has at least two critical periods, one at the age of about four days and the other at about nineteen days (Payne, 1919). Under optimum humidity, an average of about 3 per cent mortality at the first critical period, and 19 per cent at the second, has been found (Romanoff, 1930 c).

Extreme humidity. Under either extreme of humidity, as shown in table 4, the mortality of the embryos remained close to normal up to the second critical period. At that time the mortality was heavy, particularly under high humidity. Many chicks failed either to break the shell, or, after breaking it, to emerge from the shell.

TABLE 4. MORTALITY OF THE CHICK EMBRYO UNDER EXTREME HUMIDITY

Stage of incubation (Days)	Percentage of mortality*	
	High humidity (Per cent)	Low humidity (Per cent)
1-5.....	3.47	1.39
5-6.....		0.75
6-7.....	0.87	
7-8.....	0.94	
8-9.....		0.86
9-10.....	2.06	
10-11.....		
11-12.....	1.16	
12-13.....		1.01
13-14.....		
14-15.....		1.11
15-16.....		
16-17.....		
17-18.....		
18-19.....	8.62	6.85
19-20.....	61.22	25.00
20-21.....	5.93	5.81
Total.....	84.27	42.78

* Expressed in percentage of eggs remaining in the incubator on successive days.

Indirect influence of humidity

Physical changes in eggs

Optimum humidity. The most evident physical change in incubated eggs is loss of weight, which was observed as early as 1825 (Prévost and Dumas). This phenomenon is due largely to the evaporation of moisture from the egg contents, and is easily detected in candling by an increase of the air cell (table 5).

Fertile eggs, besides the evaporation, have an additional loss, due to the excretion of carbon dioxide and possibly some other gases as by-products of the embryonic metabolism. Our data show a distinct difference in the loss of weight in fertile and in infertile eggs under optimum humidity (figure 8). Incidentally, this difference is very close to the theoretical amount of the excreted carbon dioxide; yet there must be some gain in fertile eggs because of oxygen absorbed by the embryo. It is possible that fertile eggs lose more moisture than do infertile eggs, and also lose some unaccountable gaseous matter, especially at the latter part of the incubation.

TABLE 5. PHYSICOCHEMICAL CHANGES IN EGGS AND EGG CONTENTS UNDER OPTIMUM HUMIDITY

Stage of incubation	Size of air cell (in diameter)	Loss of weight in eggs		Dry-matter content		
		Fertile eggs	Infertile eggs	In yolk	In albumen	In shell
(Days)	(Centimeters)	(Grams)	(Grams)	(Per cent)	(Per cent)	(Per cent)
0.....	1.7			52.84	11.40	78.88
1.....	1.9	0.402		51.56	11.19	81.58
2.....	2.1	0.403		50.59	12.64	82.88
3.....	2.2	0.368		50.53	14.50	85.26
4.....	2.5	0.428		49.95	18.83	85.56
5.....	2.6	0.407		46.90	23.79	86.40
6.....	2.6	0.471	0.398	45.98	31.18	89.51
7.....	2.7	0.412	0.414	43.95	35.23	88.46
8.....	2.8	0.463	0.432	46.45	40.11	89.27
9.....	2.9	0.438	0.459	44.67	37.10	88.46
10.....	2.9	0.474	0.469	45.00	37.14	89.28
11.....	3.0	0.475	0.465	47.90	42.02	89.61
12.....	3.1	0.519	0.475	45.74	44.09	88.56
13.....	3.1	0.548	0.476	48.90	38.60	87.79
14.....	3.2	0.558	0.499	42.50	39.20	88.59
15.....	3.2	0.494	0.490	48.77	38.29	88.79
16.....	3.4	0.563	0.485	52.60	33.85	89.20
17.....	3.4	0.565	0.476	52.16		88.82
18.....	3.6	0.558	0.470	47.95		87.61
19.....	3.7	0.586	0.462	54.96		86.40
20.....	3.9	0.583	0.475	58.07		88.60
21.....			0.481	54.99		94.17
Total { grams.....		9.72	9.43			
per cent.....		15.92	15.49			
Average.....		0.486	0.449	49.23	29.95	87.44

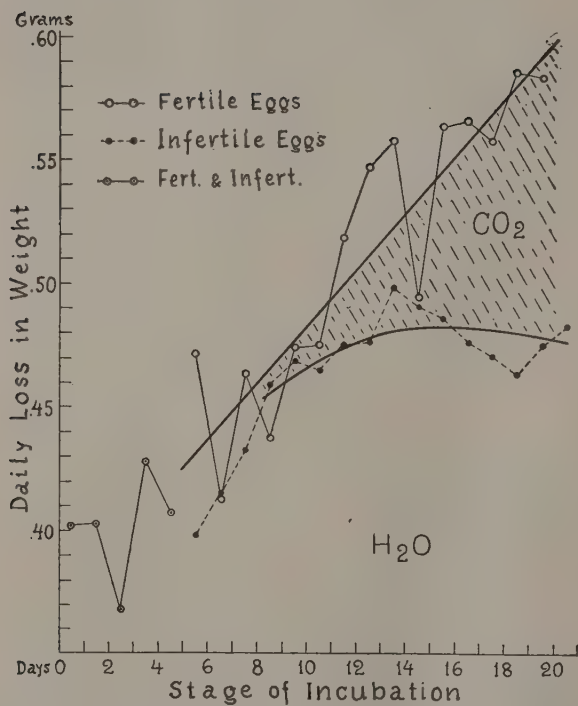


FIGURE 8. DAILY LOSS IN WEIGHT BY FERTILE AND BY INFERTILE EGGS UNDER OPTIMUM HUMIDITY

Extreme humidity. There is a considerable difference in the loss of weight in incubated eggs under high and under low humidity, this loss being, respectively, 13.20 and 18.47 per cent of the total weight (table 6). Under high humidity, the infertile eggs seemed to lose more weight, in relation to the fertile eggs, than would be expected (figure 9).

TABLE 6. PHYSICOCHEMICAL CHANGES IN EGGS AND EGG CONTENTS UNDER EXTREME HUMIDITY

Stage of incubation	Size of air cell (in diameter)		Daily loss of weight in eggs				Dry-matter content			
	High humidity	Low humidity	High humidity		Low humidity		High humidity		Low humidity	
			Fertile eggs	Infertile eggs	Fertile eggs	Infertile eggs	In yolk	In albumen	In yolk	In albumen
(Days)	(Centimeters)	(Centimeters)	(Grams)	(Grams)	(Grams)	(Grams)	(Per cent)	(Per cent)	(Per cent)	(Per cent)
0.....	1.7	1.7	0.265		0.484		52.84	11.40	52.84	11.40
1.....	1.8	1.9	0.276		0.311		51.14	12.05	51.59	12.41
2.....	2.0	2.3	0.404		0.406		49.58	11.74	51.38	12.40
3.....	2.2	2.4	0.403		0.510		48.51	13.37	48.90	13.35
4.....	2.3	2.5	0.301		0.446		46.26	15.75	47.99	15.91
5.....	2.3	2.5					46.34	28.13	46.91	25.30
6.....	2.5	2.8	0.218	0.298	0.518	0.554	42.42	29.95	45.18	30.88
7.....	2.5	2.8	0.314	0.329	0.574	0.600	43.62	33.46	46.48	37.39
8.....	2.6	2.9	0.314	0.331	0.475	0.580	44.98	35.67	44.25	38.04
9.....	2.8	3.0	0.352	0.333	0.584	0.580	43.07	37.76	44.46	39.03
10.....	2.8	3.0	0.390	0.325	0.558	0.610	45.43	39.14	45.09	43.48
11.....	2.8	3.2	0.270	0.338	0.564	0.600	46.44	38.81	47.83	44.07
12.....	2.8	3.2	0.409	0.358	0.607	0.590	44.16	40.07	47.53	43.34
13.....	2.9	3.2	0.428	0.375	0.618	0.660	45.78	40.82	46.86	42.10
14.....	3.0	3.2	0.495	0.398	0.594	0.692	45.11	38.36	47.05	38.49
15.....	3.1	3.3	0.603	0.396	0.665	0.680	48.68	35.73	48.16	35.20
16.....	3.1	3.3	0.473	0.413	0.615	0.712	52.00	33.71	51.27	35.66
17.....	3.2	3.4	0.474	0.412	0.564	0.632	41.61		54.19	
18.....	3.3	3.5	0.557	0.399	0.684	0.652	46.03		52.26	
19.....	3.7	3.8	0.549	0.393	0.793	0.653	39.76		49.09	
20.....	3.7	4.2	0.544	0.400	0.677	0.630	38.13		47.79	
21.....				0.346		0.654	45.81		61.76	
Total {	grams.....		8.04	7.49	11.25	12.24				
per cent.....			13.20	12.30	18.47	20.10				
Average.....			0.402	0.357	0.562	0.583	45.80	29.17	49.04	30.50

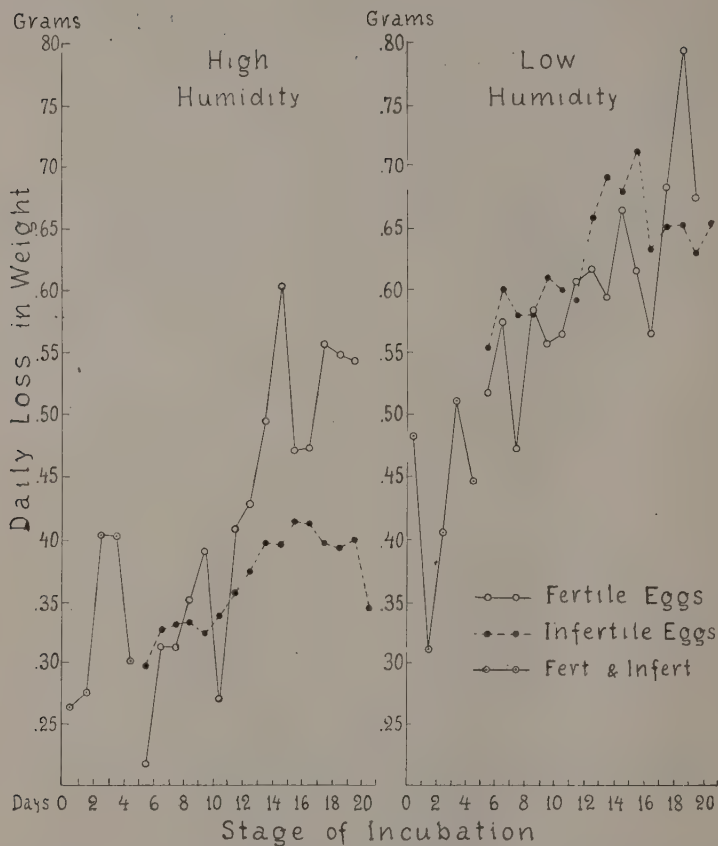


FIGURE 9. DAILY LOSS IN WEIGHT BY FERTILE AND BY INFERTILE EGGS UNDER EXTREME HUMIDITY

Chemical changes in egg contents

Optimum humidity. Among the chemical changes noted in the contents of incubated eggs is the amount of moisture, particularly in yolk and albumen (table 5). The albumen at the beginning loses moisture in two ways: in evaporation through the eggshell, and in diffusion through the vitelline membrane into the yolk. Therefore the yolk gains slightly in moisture content (figure 10). After about twelve days of

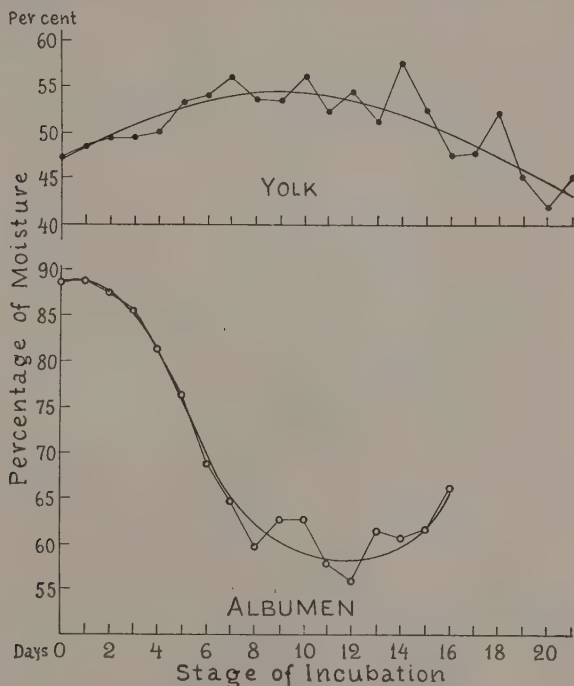


FIGURE 10. CHANGES IN THE MOISTURE CONTENT OF YOLK AND OF ALBUMEN IN FERTILE EGGS UNDER OPTIMUM HUMIDITY

incubation this process reverses. The yolk loses moisture, while the albumen rapidly gains moisture until the time of its final disappearance.

The eggshell and the shell membranes are subjected but little to the change of moisture content during the incubation period. It was found

(table 5) that the moisture was high at the beginning, and remained almost constant until the hatching time, when the eggshell became noticeably dry and brittle.

Extreme humidity. As is shown in table 6 and graphically illustrated in figure 11, the moisture content of yolk and of albumen under

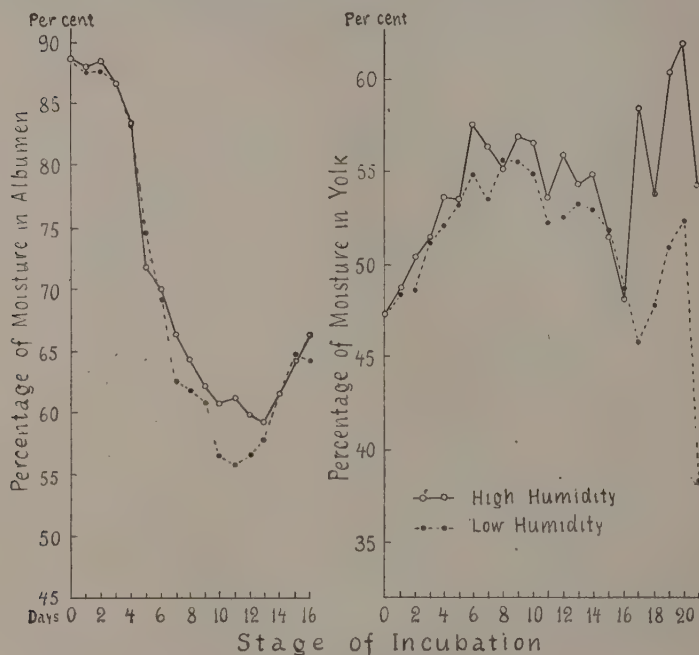


FIGURE 11. CHANGES IN THE MOISTURE CONTENT OF YOLK AND OF ALBUMEN IN FERTILE EGGS UNDER EXTREME HUMIDITY

the two extreme conditions of humidity are somewhat different. High humidity produces a watery condition, and low humidity a dried condition, of these egg constituents, particularly of the yolk. For example, the density of yolk and of albumen of infertile eggs incubated for twenty-one days was found to be 1.03363 and 1.05100 under high humidity, and 1.03640 and 1.05898 under low humidity, respectively (table 7). The amount of total ash and of calcium in the yolk sac

TABLE 7. DENSITY OF YOLK AND OF ALBUMEN OF INFERTILE EGGS UNDER OPTIMUM AND UNDER EXTREME HUMIDITY

	Number of observations	Density	
		Yolk	Albumen
Fresh eggs.....	5	1.03073	1.03958
Incubated eggs (21 days):			
High humidity.....	16	1.03363	1.05100
Optimum humidity.....	12	1.03567	0.05330
Low humidity.....	5	1.03640	1.05898

during the last week of incubation did not show any apparent difference under either extreme humidity condition.

Shell membranes, as well as other egg constituents, were found at the time of hatching to be more wet under high humidity, and more dry under low humidity, than under the optimum condition.

DISCUSSION

Dareste (1891) stated that the chick embryo can grow normally within a range of from 40 to 80 per cent relative humidity. Our data show that the embryo may grow at a humidity of 40 or 80 per cent without any sign of abnormality, but up to the fourteenth day of incubation only. From that stage the embryonic development is noticeably disturbed. The first indication of this disturbance is seen in the irregularity of growth (figure 4) and in the completæ destruction of normally observed growth cycles (figure 7). On the other hand, the assimilation of calcium from the eggshell is greatly favored by high humidity (figure 5). This indicates that it is possible, by the proper use of high humidity in the incubator during certain stages of the embryonic development, to produce a healthier chick, with stronger skeletal structure, and possibly more resistance to some ailments in the brooding period such as rickets or leg weakness, than is produced under the modern practice of artificial incubation.

In these experiments, the embryos presumably became weak on reaching the second critical period, at about nineteen days of incubation, and they could not overcome even slightly abnormal conditions developed within the egg. Under either extreme of humidity many of the embryos died after pipping—that is, after breaking the inner shell membrane. It is evident that the trouble comes when the embryo is ready to use its lungs and when it is entirely dependent on the environment. Some embryos succeeded in making a hole in the eggshell, and, with the beak partly outside, were unable to free themselves and died.

The idea of Réaumur (1749), supported at present by practical men engaged in artificial incubation, was that under high humidity embryos die from suffocation caused by watery albumen. So far as we know, there is no albumen, as such, in an egg at hatching time, because it has

already been utilized. Possibly the contents of the allantoic sac have been taken for the albumen. Our observations show that suffocation of the embryo, which presumably is already weakened and of low vitality, has been caused either by wet shell membranes or by wet contents of the allantoic sac, or by both.

On examination of the dead embryos, we frequently found also that the yolk sac had not been completely indrawn, or that the contents of the yolk were watery, or, in a few instances, that the yolk sac was ruptured. From this we are inclined to believe that the physicochemical condition of the contents of the yolk sac plays an important rôle in embryonic development, particularly during the second critical period of incubation. Any changes in the yolk sac resulting from factors in the environment, such as humidity, undoubtedly affect the regulation of nourishment and the metabolic processes of the embryo. This is soon shown, in some naturally susceptible individuals, by the lowering of vitality and by the development of pathological conditions leading to death.

From the foregoing it is seen that the influence of humidity on the chick embryo in general is both physiological and physical. Through the change in the physicochemical constitution of the contents of the yolk sac — the main source of the embryonic nutrition in the latter part of the development — it disturbs the metabolism, as may be indicated by growth, vitality, and susceptibility of the embryo to an environment. Through the change in the amount of water in the contents of an egg, particularly in the linings surrounding the embryo, it makes an obstruction to hatching, by the restriction of free movement and by the hindrance to pulmonary respiration. Thus the most naturally susceptible embryos die first; afterward the mortality increases as the humidity goes toward the high or the low extreme.

SUMMARY

From the experimental data it is concluded that the extreme conditions in the incubator, 80 and 40 per cent relative humidity, have both direct and indirect influence on the developing chick embryo, as follows:

I. The direct influence:

1. The growth of the embryo was rather irregular, being somewhat hastened at high and retarded at low humidity.
2. The cycles in the growth of the embryo were decidedly disturbed.
3. The calcium metabolism in certain stages of the embryonic development was much better at high than at low humidity.
4. The mortality of the embryo was noticeably increased during the second critical period — at the nineteenth day of incubation — particularly at high humidity.

II. The indirect influence:

1. The physicochemical constitution of the yolk sac was slightly changed; this might have affected the embryonic metabolism, leading to the embryo's low vitality and its susceptibility to environment.

2. The water content of the shell membranes and the contents of the allantoic sac corresponded to the humidity condition; this might have made an obstruction to hatching through the restriction of free movement of the embryo and through the hindrance to pulmonary embryonic respiration.

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